Aus der Medizinischen Universitätsklinik (Direktor Prof. F. Koller)
und aus der Chirurgischen Universitätsklinik
(Direktor Prof. R. Nissen), Basel

Blood Coagulation Studies and Extracorporeal Circulation in Man

INAUGURAL-DISSERTATION
zur Erlangung der Doktorwürde der gesamten Heilkunde,
vorgelegt der Medizinischen Fakultät der Universität Basel

von
NIKOLAUS THURNHERR
von Au (St. Gallen) und Basel

1966

F. K. SCHATTAUER-VERLAG · STUTTGART 1967

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Von der Medizinischen Fakultät genehmigt
auf Antrag von Prof. F. Koller

Tag der Promotion: 28. Oktober 1966

UNIV.
LEIDEN
BIBL.

Sonderdruck aus Thrombosis et Diathesis haemorrhagica
Band XVIII, 1967

Many authors have studied the alterations of blood coagulation during extracorporeal circulation (ECC). However a large number of the reports are limited to a few clotting parameters only, and in most cases the priming of the pump oxygenator is not taken into consideration for the early changes in the blood coagulation system. It is valuable therefore, to investigate on a large basis patients undergoing ECC.

The investigations of blood coagulation are complicated by the variations which occur during ECC. Hence it is our purpose to characterize the »normal« changes during open heart surgery thus making it easier to detect the »abnormal« cases.

For the comparison of the results obtained from our patients, the determinations have been planned in function with the successive phases of the operation and thus any change may be related to the corresponding operation phase. Moreover, the dosage and elimination rate of heparin have been taken into account, because it was uncertain if the amount of heparin administered was adequate.

Prior to operation the investigated cases are controlled for hemorrhagic diathesis. A further control is made during the operation, shortly before bypass, i. e. after the first surgical phase, which perhaps alters the blood coagulation system. Following the mixing of the patients blood with the priming of the pump oxygenator, the next analysis takes place.

After 1 hr of perfusion the fourth analysis is performed. Every subsequent hour of prolonged perfusion is followed by a control.

A further control is made, when the patient leaves bypass, 10–15 min after the administration of protamin chloride for heparin neutralization. The last control on the day of operation follows 3 hrs later.

It is insufficient to know the absolute result of blood coagulation analysis without knowing the conditions of perfusion (i. e. contents and volume of priming).

With a differentiated analytical system we get more exact information on blood clotting disorders, which enables us to direct an effective therapy.

Materials and Methods

We have controlled 18 adults and 7 children subjected to open heart surgery with ECC, at the Bürgerspital, Basle (Switzerland). Amongst these patients, 12 suffered from congenital heart failure and 13 had acquired valvular diseases.

Oxygenators

During this study 2 different types of bubble-oxygenators have been employed. The first machine works with 3 DeBakey-roller-pumps (1) and the system of tubes is of Tygon-material.

The second oxygenator is also a bubble-type-model, the Ryggs-Kyvsgaard MK IV, but with only one roller-pump and the system of tubes made of silicone rubber. The defoaming material of both machines is of silicone and the metal connectors are of highly polished steel. The perfusion rate was 1.8–2.5 l/m2 body surface/min. Perfusions were mostly carried out at moderate hypothermia (30–31° C). The lowest temperature ever obtained was 21° C (1 case). The maximum temperature difference between patient and heat exchanger was 11° C. The minimum and maximum time of perfusion was 17 min and 420 min, respectively.

Dosage of Heparin

Administration of heparin and neutralization of heparin with protamin chloride.

Before inserting the cannulae into the great vessels, the patient receives 3 mg (300 IU) of Heparin (Vitrum)/kg body weight. A further dose of 1.5 mg heparin/kg body weight is administered should the perfusion exceed 2 hrs. The donor's blood contains 20 mg heparin/450 ml blood. Protamin chloride (Protamin® 5% Roche) is administered proportionally to the initial heparin dose (in relation to body weight) using a coefficient of 2.4. The heparin administered during perfusion is not taken into account.

Donors' blood

The donors' blood which is required to prime the oxygenator, is drawn 1–3 hrs in advance. For 450 ml of blood, one uses 20 mg Heparin (Vitrum) in 20 ml of 0.9% sodium chloride.

Blood sample

Before and after perfusion blood samples are taken by a catheter from the radialis artery of the patient. During bypass, blood samples are taken directly from the arterial reservoir of the oxygenator. All analyses were carried out in oxalated blood. During the perfusion Hexadimethrine Bromide (Polybrene® Abbott) was added to neutralize the heparin in order to determine the factors II, V, VII, VIII, X and fibrinogen.

For the purpose of measuring the serum prothrombin and factor IX, only Polybrene® was added to the blood of the oxygenator, whilst remaining controls were executed in heparinized blood.

Oxalated blood: 1 part of 0.1 m sodium oxalate, 9 parts of blood.

Blood of the oxygenator with the addition of Polybrene®: 10 ml of oxalated blood of the oxygenator, 0.125 mg Polybrene® (Abbott) dissolved in 0.25 ml 0.9% sodium chloride.

Serum: Blood is collected in glass tubes and stored at room temperature. During perfusion one adds 10 ml blood of the oxygenator to 0.125 mg Polybrene R, dissolved as above.

Priming

The greater part of the priming consists of heparinized donor blood. As plasma expander a low molecular dextran (Rheomacrodex®) (Pharmacia) or a product of depolymerized gelatine (Haemaccel®, Behring) is used. In addition there are: Human albumin, bicarbonate ($NaHCO_3$) 5% and sodium chloride 0.9%.

For a patient's blood-volume of 5 l the priming contains:

500 ml plasma expander
150 ml human albumin
3150 ml heparinized donor blood
350 ml $NaHCO_3$ 5%
500 ml sodium chloride 0.9%.

Estimation of coagulation factors

Factor II and VII determination: Koller et al. (2).
Factor X determination: Bachmann et al. (3).
Factor V determination: Kappeler (4).
Fibrinogen level: Clauss (5).
Time of recalcification, thrombin time, serum prothrombin (6).
Thrombocyte count: Nygaard (7).

Factor VIII and IX: Geiger (8).
Whole blood clotting time: Lee and White.
Bleeding time: Yvy.
Blood thromboplastin generation test: Duckert et al. (9).
Serum hemoglobin (10).
Determination of the heparin level in plasma: Modification of the method of Blombäck et al. (12).
Euglobulin lysis time: Milstone (13).
Clot retraction according to Saeman (unpublished data): Oxalated plasma rich in thrombocytes (obtained by sedimentation of erythrocytes) is clotted with $CaCl_2$ in a glass tube. Incubated at 37° C the clot within an hour retracts normally to half its original length, which is considered to be the 100% value, whilst thrombocytopenia and thrombocytopathia slow down or decrease the retraction.

Results

The same designation for blood samples is adopted for all patients at the time of blood sampling (see Table 1) and is always used in the following graphs.

Table 1. Blood Samples.

1 a	1 or several days before operation.
P 1	After thoracotomy, a short time before heparinizing the patient, i. e. shorthly before starting ECC (operative stress).
M 2	Priming-blood
M 3	10–15 min after beginning of ECC i. e. after the total mixing of the patient's and the machine blood.
M 4	50–60 min after start of ECC.
M 5 – M X	2 or several hrs of bypass.
P 5	Patient's blood 10–20 min after neutralization of heparin with protamin chloride.
P 7	2–3 hrs after neutralization.
P 8	12–18 hrs after neutralization.
P 9	24 hrs after P 8.
P10	24 hrs after P 9 and so on.

For the interpretation of the clotting factor activity (% of the norm) it is important to know the volumes of both, patient's blood and priming, and their respective activities; only then is it possible to differentiate between a simple dilution and a denaturation of blood clotting factors by combining the patient's blood with the priming.

As a measure of the dilution the protein concentration is controlled. Assuming 100% is in P 1, only 68% is found in M 3. Since the priming contains proteins lacking in clotting activity the value of the clotting factor activity expected in M 3, without any inactivation by ECC, must be below 68%.

In vitro experiments have shown, that the clotting factor activity measured in heparinized blood immediately after neutralization with Polybrene® is almost similar to the values obtained before heparinization.

Quick Values and Extrinsic System

Quick values and quantitative assays of factors II, V, VII and X and fibrinogen have been performed after heparin neutralization with Polybrene when using the oxygenator's blood. Values obtained in heparinized blood are useless (14).

Figs. 1–5 show that the Quick value and the values of the extrinsic factors activity at the beginning of the perfusion (M 3) and after 1 hr (M 4) are obviously below the levels found before operation. During perfusion these factors remain nearly constant.

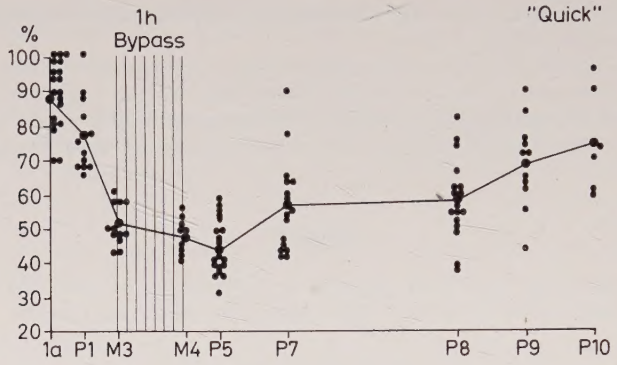

Fig. 1. "Quick-Value" during ECC. See also Table 1.

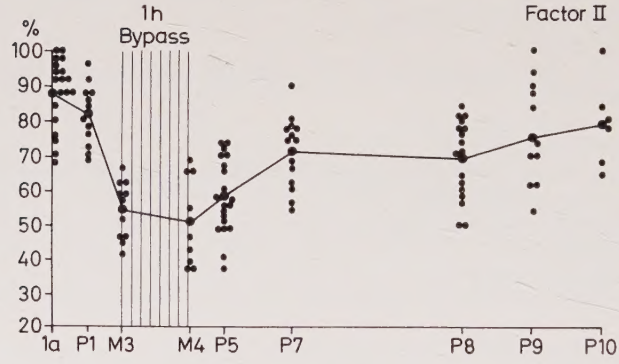

Fig. 2. Factor II determination during ECC. See also Table 1.

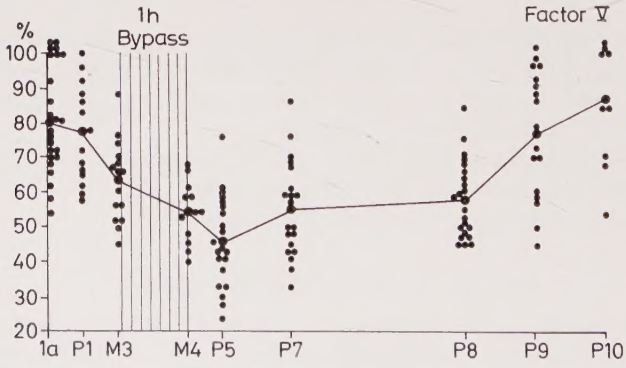

Fig. 3. Factor V determination during ECC. See also Table 1.

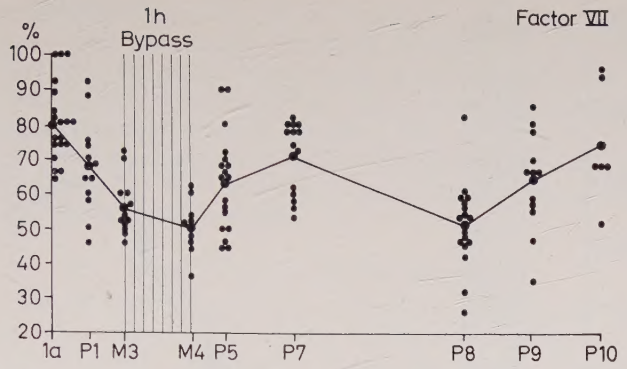

Fig. 4. Factor VII determination during ECC. See also Table 1.

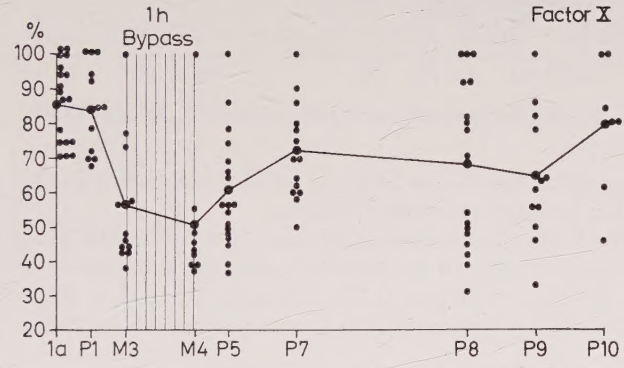

Fig. 5. Factor X determination during ECC. See also Table 1.

The levels normalize on the second or third postoperative day. Patients, who preoperatively have been anticoagulated orally, once again, on the second or third postoperative day showed a decrease of the vitamin K_1 depending factors.

During long perfusion and also after perfusion the strongest depression is seen in factor V activity. This also is the only factor of the extrinsic system that reaches its lowest values after neutralization with protamin chloride (15). In vitro heparinized and with Polybrene® neutralized blood shows a significant inactivation of factor V activity after neutralization.

Fibrinogen

With a mean value of 230 mg% the fibrinogen content already drops during the thoracotomy, to a mean value of 195 mg%. The priming contains about 100 mg% fibrinogen, so that M 3 with a mean value of 150 mg% results only by mixing the blood of the patient with the priming, assuming, of course, the blood volume of the patient is about equal to that of the priming. During perfusion the fibrinogen level stays nearly unchanged (132 mg%) (Fig. 6).

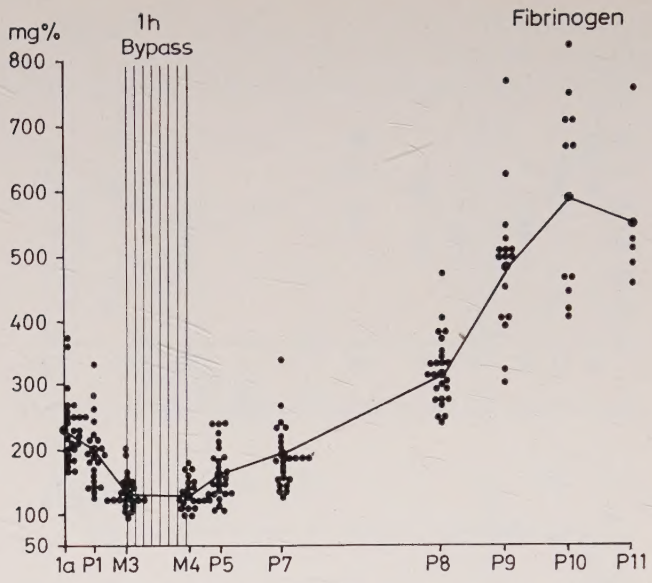

Fig. 6. Fibrinogen level during and after ECC. See also Table 1.

The theoretical fibrinogen levels in M 3 and M 4 calculated from P 1 and M 2 are in good correlation with the determined values.

In P 8, i. e. 12–18 hrs after perfusion already, the mean value is higher (320 mg%) than the preoperative value. In all observed cases the fibrinogen concentration rises promptly after the neutralization of the circulating heparin. One observes on the second, third and fourth postoperative days a considerable hyperfibrinogenemia.

Euglobulin Lysis Time

When applying the method of Milstone (13) the inhibitors of the fibrinolytic system are almost eliminated, so that it is already possible to seize a minimum fibrinolytic activity. A lysis time of over 2 hrs reflects a normal fibrinolytic activity. At fibrinogen levels around 100 mg% the fibrinolytic activity is of clinical importance only if the euglobulin lysis time is shorter than 15 min. None of the 24 cases investigated preoperatively (1 a) as well as 2–3 hrs after completion of bypass (P 7), showed an abnormal fibrinolytic activity (Fig. 7).

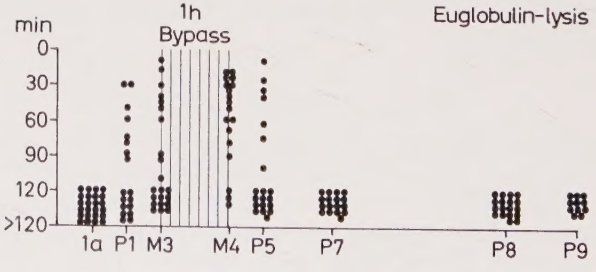

Fig. 7. Euglobulin lysis time during ECC. See also Table 1.

During the time of operation, but before bypass, 8 patients showed a slight fibrinolytic activation, which moderately increased during ECC. After the first hour of perfusion 18 out of 21 showed an increase in fibrinolytic activity. Ten min after neutralization (P 5) only 2 patients continued to show an increase in lytic activity. After a further 3 hrs all patients were found to be normal (30).

Cases with elevated fibrinolytic activity never showed an excessive depletion of fibrinogen or a sudden drop in the number of platelets (18). It was never necessary to use inhibitors in order to stop fibrinolysis.

Platelet Counts and Clot Retraction

Fig. 8 shows the immediate drop of thrombocytes to 20'000/mm³ blood at the start of the perfusion. The values remain nearly constant throughout the perfusion even when prolonged. After ECC the number of platelets quickly increases to 50'000/ml blood. This is followed by another slight drop on the second postoperative day (19, 20). Normalization of the platelet count is observed on the $7^{th}-10^{th}$ postoperative day. Clot retraction is found almost normal after completion of the perfusion (P 5, P 7) but becomes increasingly abnormal in the following days. In other words during the first, second and third day after perfusion the circulating platelets are unable to make a clot retract.

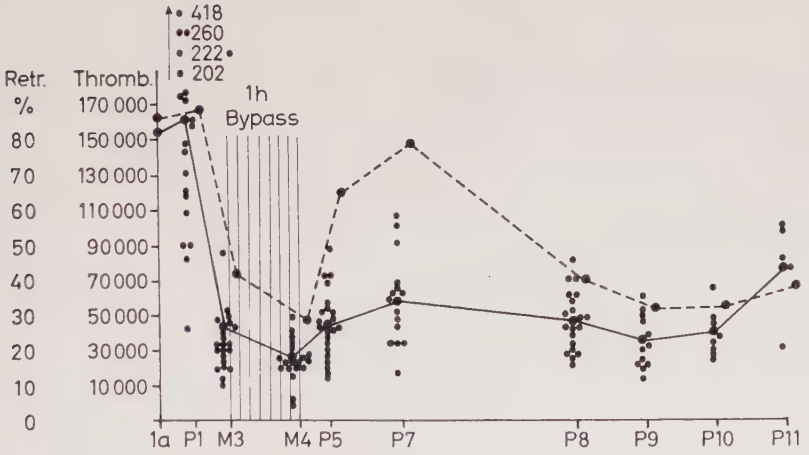

Fig. 8. Platelet counts and clot retraction during ECC. —— Thrombocytes, – – – Clot retraction. See also Table 1.

Serum Prothrombin, Factor VIII (AHG) and Factor IX (Christmas Factor)

The serum prothrombin (normal range of the method 1–8%) is normal before ECC, but sometimes increases to an abnormal value during bypass. This might be explained by the addition of Polybrene® which is needed for the neutralization of heparin in the blood of the oxygenator to make it clot. By itself, Polybrene, when added in excess, is able to produce an abnormal serum prothrombin. It is evident, that after neutralization with protamin chloride nearly all observed cases show abnormal values of serum prothrombin (P 5). Two – three hrs after neutralization (P 7), the greater part of the investigated cases still show an abnormal result of serum prothrombin.

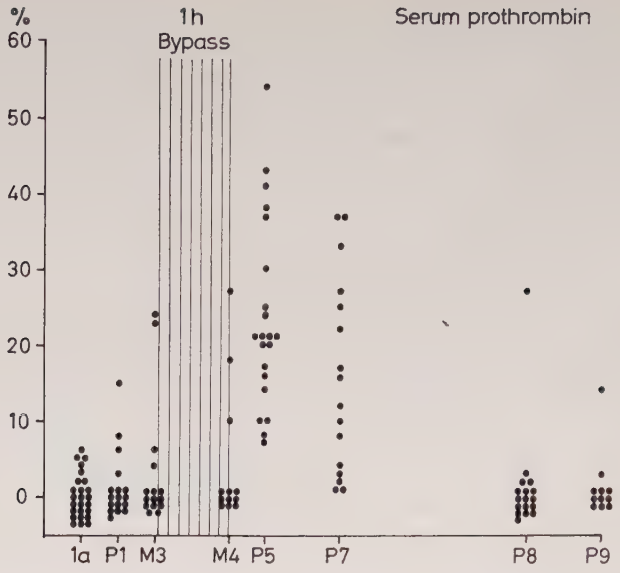

Fig. 9. Serum-prothrombin and ECC. See also Table 1.

The following day in P 8 all cases returned to normal values (Fig. 9). The question now arises as to whether the abnormal serum prothrombin is due to a denaturation of a blood clotting factor (factor VIII, or IX) or whether it is caused by an unspecific effect of protamin chloride. Perkins (21) observed a depletion in factor VIII, an abnormal serum prothrombin and a pathological thromboplastin generation, when he administered a high dose of protamin chloride to dogs. According to this investigations the alterations depend on the dose and infusion rate of protamin chloride.

On the contrary we were unable to find a correlation between pathological serum prothrombin, factor VIII (AHG), factor IX (Christmas factor), factor V and factor X (Table 2). Besides, there was no significant disturbance in the thromboplastin generation. Considering, that at the end of the perfusion the blood of the pump – oxygenator is nearly identical to that of the patient in P 5, we have tried to obtain new information by adding progressive doses of protamin chloride in vitro to the blood of the machine. However, neither the in vivo nor the 10 times higher concentrations could produce an elevated serum prothrombin (21). The minimum activity of factor VIII

Table 2.

		P 1	P 5	P 8
Serum prothrombin	%	1,7	25,6	1,7
		(7)	(7)	(7)
Factor VIII (AHG)	%	89	73	100
		(5)	(5)	(5)
Factor IX (Christmas factor)	%	89	85	79
		(7)	(7)	(7)

Mean values of serum prothrombin, factor VIII (AHG) and IX (Christmas factor) of 7 patients, wherein all showed abnormal serum prothrombin in P 5 and P 7. ( ) = number of patients.

(AHG) was found in the priming, but this activity was far below the value expected merely by dilution.

Thrombin Time Following the Neutralization of Heparin with Protamin Chloride

For heparin neutralization after bypass the patient receives protamin chloride 2.4 times the weight of the initial heparin dose (= 7.2 mg protamin chloride /kg body weight).

The 5% solution of protamin chloride was diluted in 50 cm^3 of 0.9% sodium and infused within 10–20 min in order to avoid a decrease of the blood pressure and a bradycardia. This is a rather high dose in comparison to the data of the literature. Under these conditions we did not find a heparin rebound even when applying a sensitive method for the detection of heparin (22, 23). The thrombin time was normalized with protamin chloride in all cases (18).

Heparin Elimination Rate during ECC

We obtained a rather constant heparin concentration at the beginning of the perfusion (M 3), when we administered 3 mg/kg body weight of heparin (300 IU Heparin) and an additional dose of 20 mg heparin/450 ml donors'blood.

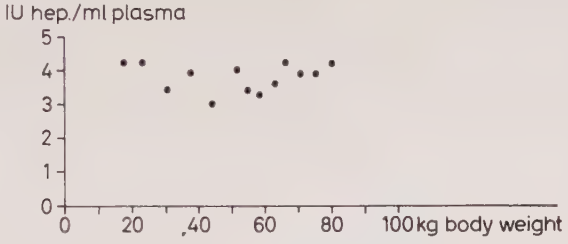

Fig. 10. Heparin levels in plasma at the beginning of the perfusion in M 3 plotted against body weight.

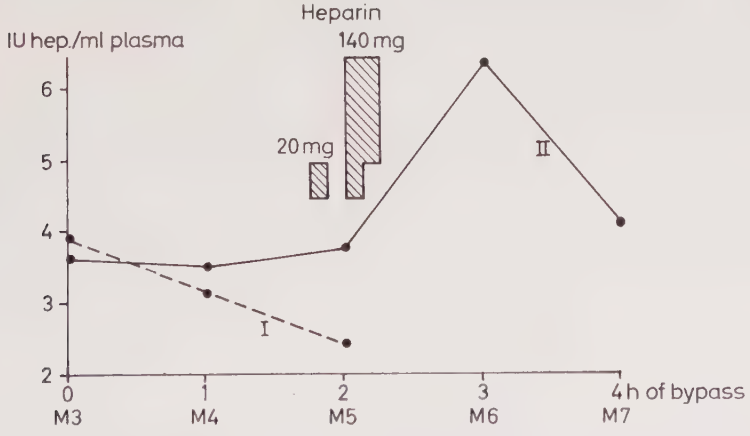

Fig. 11. Heparin levels during ECC. Case 1: Without further heparin during ECC. Case 2: With additional heparin during ECC.

Fig. 10 shows the initial plasma heparin levels independent of body weight and the obtained mean value of heparin concentration in plasma (3.75 IU/ml plasma = 37.5μg).

It is well known, that the heparin elimination rate varies in individuals (20, 24, 29).

However, to ascertain the elimination rate of heparin, we had to investigate cases with a long perfusion time but without any further administration of heparin during bypass.

Within 2 hrs we observed a fall in the heparin concentration to about half the initial value (Fig. 11, Case 1).

Fig. 11 shows the heparin levels during a 4 hr perfusion (Case 2) where after 2 hrs the patient received half of the initial dose of heparin. Consequently, at the end of the perfusion the heparin concentration is nearly identical to the initial heparin level.

Hemolysis in Function with the Perfusion Time (Serum Hemoglobin)

Hemolysis varies in strength in patients, who have experienced perfusions of similar lengths. However, in a single patient hemolysis is rather proportional to the duration of perfusion. In 14 patients the mean value is 44 mg% serum hemoglobin after 10 min of perfusion. After 60 min the mean value is already 86 mg% in 13 patients (Fig. 12).

In all patients hemolysis has disappeared after 18 hrs.

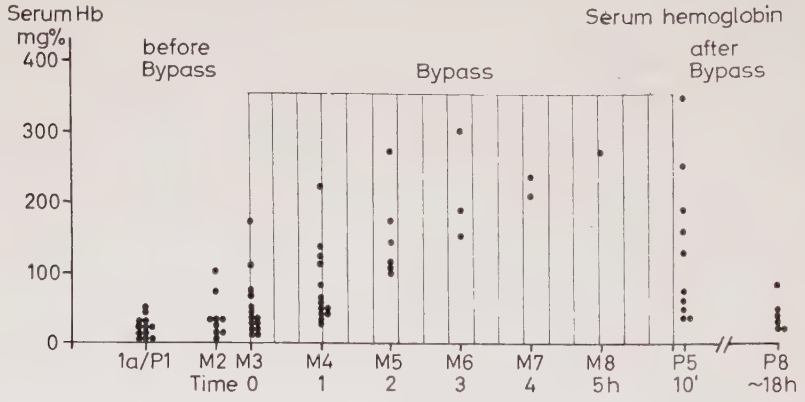

Fig. 12. Hemolysis in function with the perfusion length.

Discussion

Bleeding occurs more frequently after a long lasting perfusion. Regarding clotting our findings showed no differences between the two types of pump-oxygenators. There is a large number of reasons for blood clotting abnormalities and disturbance of hemostasis and therefore a well constructed system of analysis is required.

The results of the laboratory test shown in the graphs should not be taken as absolute values. It is important to take into account all the conditions capable of altering the results. Once again, we must point out that a decrease in clotting factors activity is brought about already by a simple dilution of the patient's blood (P 1) with the priming (M 2). Further, it must be remembered that the analysis of the factors of the extrinsic system are performed in heparinized blood, to which Polybrene® is added for neutralization. Although the results are almost identical to those found in blood without heparin, experiments in vitro with Polybrene show that the values for

factors II, V, VII and X in neutralized heparin blood are slightly diminished in comparison to those prior to heparinization.

Throughout the perfusion there is a decrease in the »Quick«-value, in factors II, V, VII and X. During bypass the most essential depletion is found in factor V. All these factors, however, exist in concentrations that allow normal coagulation.

The fibrinogen levels are practically constant during bypass. On completion of the perfusion the fibrinogen levels are only about 60% compared with the preoperative value, but after a few hours these levels revert to normal.

Nevertheless, it is possible that this transitory hypofibrinogenemia associated with the use of plasma expander causes the formation of a defective clot.

In nearly all cases after neutralization (P 5, P 7), an abnormally high serum prothrombin is found. Perkins (21) obtained the aforesaid results associated to a factor VIII decrease when administering protamin chloride to dogs. We were unable to find a correlation between a decrease in a single clotting factor (V, VIII, IX or X) and the abnormality of serum prothrombin (Table 2). Thromboplastin generation also failed to provide us with new information.

Since, during bypass the platelets are even lower in number, it is impossible to identify them as the cause of abnormal serum prothrombin in P 5. In the neutralized blood of the pump however, the prothrombin is found normal.

In vitro neutralization of the oxygenator blood with protamin chloride on completion of perfusion results in an abnormal prothrombin consumption, if neutralization is insufficient. An adequate neutralization or overdose however, will normalize serum prothrombin. Following the perfusion there is no correlation between the level of serum prothrombin and hemorrhagic diathesis. In cases of increased bleeding fraction I of Cohn [fibrinogen, factor VIII (AHG)] showed a good effect, when administered in high doses (5 10 g fibrinogen). However, it does not seem possible that the fibrinogen level is responsible for the bleeding. We are unable to explain the manner of action of this therapy. Neither the serum prothrombin nor the level of fibrinogen can predict normal hemostasis or increased bleeding in patients.

It remains undetermined, therefore, as to whether the abnormal prothrombin consumption is the result of a simultaneous decrease of several factors or an unspecific effect of protamin chloride.

All cases showed absence of fibrinolytic activity preoperatively. None of them produced later the dangerous complication of fibrinolysis or fibrinogenolysis.

At the beginning of the ECC, regardless as to which one of the two types of oxygenators is involved, the platelets drop to values of 20'000 30'000/mm^3, which appear to be below those stated in the literature (32, 19, 18 et al.). Part of the platelets adhere to the surface of the oxygenator and it is assumed, that another part forms aggregates and remain caught in the microcirculation (28). Those aggregated platelets seem to be released into circulation after completion of bypass. This could be the explanation for the sudden rise in the platelet count minutes after the completion of ECC in P 5. The number of thrombocytes is found to be nearly constant during perfusion.

In our opinion the thrombocytes counted are at least partially intact, since they provoke a clot retraction. Following an operation the lowest value of the thrombocytes is found on the 2nd postoperative day (19, 20). Furthermore there is no correlation between thrombocytopenia and the postoperative loss of blood (19).

As a consequence of our heparin determinations we found necessary to administer a further heparin dose half the initial one, if the perfusion exceeds 2 hrs. Under these conditions at the end of the perfusion, the heparin concentrations are about equal to the heparin level at the beginning of the bypass. This fact is of extreme importance, if

we risk to administer a standardized protamin chloride dose based only on body weight (25, 26, 27). There is no doubt that an overdose of protamin chloride may provoke hemorrhagic disorder.

In spite of extensive investigations in blood coagulation in patients undergoing ECC we are yet unable to predict from laboratory results, if the patient will be a bleeder. Hemorrhages are more common after a long duration of perfusion.

Apart from the plasmatic factors and platelets, we have also to consider vascular damage, as at this stage it is impossible to establish that one particular factor is the cause of the hemorrhagic disorder after ECC.

Our results – especially the immediate increase of platelet count and fibrinogen concentration – show that intravascular coagulation in all 27 cases of ECC investigated during one year never occurred.

Summary

Blood clotting investigations have been executed in 25 patients who have undergone open heart surgery with extracorporeal circulation. A description of alterations in the activity of blood clotting factors, the fibrinolytic system, prothrombin consumption and platelets during several phases of the operation is given.

Résumé

On a étudié le système de coagulation du sang chez 25 patients soumis à une opération à coeur ouvert avec circulation extracorporelle. On décrit les altérations des facteurs de coagulation, du système fibrinolytique, de la consommation de la prothrombine et des plaquettes en fonction des diverses phases de l'opération.

Zusammenfassung

Es werden die Gerinnungsverhältnisse bei 25 Patienten untersucht, die wegen eines Eingriffes am offenen Herzen an die Lungen-Herz-Maschine angeschlossen werden. Die Veränderungen der Aktivitäten der Gerinnungsfaktoren, des fibrinolytischen Systems, die Prothrombinkonsumption und die Thrombozyten in der jeweiligen Phase der Herzoperation werden besprochen.

Acknowledgements

I am indebted to Prof. F. Koller for having made these investigations possible in his laboratories and to Dr. F. Duckert for guidance, criticism and advice. Further I wish to thank Dres. E. Graedel, J. P. Gigon and G. Wolff for their appreciated collaboration, Miss M. Cronquist and Mrs. A. Brucker for their valuable technical assistance.

References

(1) *Yasargil, E. C.:* Eine einfache Herz-Lungen-Maschine. Thoraxchirurgie *10:* 310 (1963).

(2) *Koller, F., A. Loeliger, F. Duckert:* Experiments on a new Clotting Factor (Factor VII). Acta haemat. (Basel) *6:* 1 (1951).

(3) *Bachmann, F., F. Duckert, F. Koller:* The Stuart-Prower-Factor Assay and its Clinical Significance. Thrombos. Diathes. haemorrh. (Stuttgart) *2:* 24 (1958).

(4) *Kappeler, R.:* Das Verhalten von Faktor V im Serum unter normalen und pathologischen Bedingungen. Z. klin. Med. *153:* 103 (1958).

(5) *Clauss, A.:* Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. Acta haemat. (Basel) *17:* 237 (1955).

(6) *Duckert, F.:* Le Diagnostic des Coagulopathies. Röntgen- u. Lab.-Prax. *13:* 652 (1960).
(7) *Nygaard, K. K.:* A Direct Method of Counting Platelets in Oxalated Plasma. Proc. Mayo Clin. *8:* 365 (1933).
(8) *Geiger, M., F. Duckert, F. Koller:* Quantitative Bestimmung von Faktor VIII und IX bei Blutersippen. 5th Congr. Europ. Soc. Haematol., Freiburg (1956) p. 413.
(9) *Duckert, F., P. Flückiger, H. Isenschmidt, H. Matter, I. Vogel-Meng, F. Koller:* A Modification of the Thromboplastin Generation Test. Acta haemat. (Basel) *12:* 197 (1954).
(10) *Hunter, F. T., M. Grove-Rasmussen, L. Soutter:* A Spectrophotometric Method for Quantitating Hemoglobin in Plasma or Serum. Amer. J. clin. Pathol. *20:* 429 (1950).
(11) *Koniakowsky, L.:* Dtsch. med. Rundschau *1950:* 281.
(12) *Blombäck, B., M. Blombäck, P. Olsson, G. William-Olsson, A. Senning:* Determination of Heparin level of the Blood. Acta chir. scand. Suppl. *245:* 259 (1959).
(13) *Milstone, A.:* A factor in normal human blood, which participates in streptococal fibrinolysis. J. Immunol. *42:* 109 (1941).
(14) *Perkins, H. A., M. R. Rolfs, B. Torg:* Assay of Blood Coagulation Factors in Heparinized Blood. Thrombos. Diathes. haemorrh. (Stuttg.) *11:* 254 (1964).
(15) *Hougie, C.:* Anticoagulant action of Protamin Sulphate. Proc. Soc. exp. Biol. (N. Y.) *98:* 130 (1958).
(16) *Von Kaulla, K. N.:* Spontaneous Anticoagulants in Surgery. Acta haemat. (Basel) *24:* 135 (1960).
(17) *Phillips, L. L., J. R. Malm, R. A. Deterling:* Coagulation Defects Following Extracorporeal Circulation. Ann. Surg. *157:* 317 (1963).
(18) *Von Kaulla, K. N., H. L. Swan:* Clotting Deviations in Man during Cardiac Bypass, Fibrinolysin and Circulatory Anticoagulant. J. thorac. Surg. *36:* 519 (1958).
(19) *Schmidt, P. J., J. C. Peden, A. Brecher, A. Baranovsky:* Thrombocytopenia and Bleeding Tendency after Extracorporeal Circulation. New. Engl. J. Med. *265:* 1181 (1961).
(20) *Kendall, A. G., L. Loewenstein:* Alterations in Blood Coagulation and Hemostasis during Extracorporeal Circulation. Part I + Part II. Canad. med. Ass. J. *87:* 786, 859 (1962).
(21) *Perkins, A. H., G. Harkins, F. Gerbode, M. R. Rolfs, D. J. Acra:* Comparison of Effects of Protamin and Polybrene with Special Emphasis on the Factor VIII Deficiency Induced. J. clin. Invest. *40:* 1421 (1961).
(22) *Brögli, H.:* Der Mechanismus des sogenannten »Heparin-Rebound« nach extrakorporellem Kreislauf. Thrombos. Diathes. haemorrh. (Stuttg.) *13:* 401 (1965).
(23) *Loeliger, A.:* Diskussion zum Vortrag von *Marx, R., H. Borst,* über Heparinisierung von Patienten und Neutralisierung mit Protaminsulfat. Thoraxchirurgie *9:* 84 (1962).
(24) *Senning, A.:* Plasma-Heparin Concentration in Extracorporeal Circulation. Acta chir. scand. *117:* 55 (1959).
(25) *Perkins, H. A., J. J. Osborne, R. Hurt, F. Gerbode:* Neutralization of Heparin in vivo with Protamine; a Simple Method of Estimating the Required Dose. J. Lab. clin. Med. *48:* 223 (1956).
(26) *Hawksley, M.:* De-Heparinization of Blood after Cardiopulmonary Bypass. Lancet *I:* 563 (1966).
(27) *Andersen, H. N., M. Mendelow, N. Murray:* A comparative study of heparin neutralization and the danger of blood clotting abnormalities with protamin and polybren. J. thorac. Surg. *39:* 613 (1960).
(28) *Long, D. M., L. Sanchez, R. Varco:* The use of low molecular dextran and albumin as plasma-expanders. Surgery *50:* 12 (1961).
(29) *Wright, J. S., J. J. Osborn, H. A. Perkins, F. Gerbode:* Heparin Levels During and After Hypothermic Perfusion. J. cardiovasc. Surg. *5:* 244 (1964).
(30) *Anderssen, M. N., M. Mendelow:* Fibrinolysis During and After ECC. Arch. Surgery *86:* 649 (1963).
(31) *Holemans, R., C. Vermylen, M. Verstraete:* Disappearance of Factor V Activity after Incubation of Plasma with Heparin or Heparinoids. Thrombos. Diathes. haemorrh. (Stuttg.) *4:* 303 (1960).
(32) *Salzman, E. W.:* Blood Platelets and Extracorporeal Circulation. Transfusion *3:* 274 (1963).